Differential Diagnosis of Primary Versus Metastatic Pulmonary Adenocarcinomas Using Gene Mutation Analyses

A Case Report

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Abstract: A 61-year-old Japanese woman underwent a partial mastectomy for cancer of the right breast (pT1cN0M0, stage I). Eight months later, chest computed tomography revealed two small nodules in the left lower lobe (Segmentum basale laterale and Segmentum basale posterius; S9 and S10). She thereafter underwent partial pulmonary resections for both diagnostic and treatment purposes. The nodule of S10 was pathologically diagnosed to be primary lung cancer. The nodule of S9 was pathologically diagnosed to be poorly differentiated adenocarcinoma. The same pattern of the distribution of the p53 mutation was observed in the DNA samples of the S9 nodule and the treated breast cancer. We therefore finally diagnosed the S9 nodule to be metastatic pulmonary carcinoma. A mutation analysis of the p53 gene is thus considered to be a good modality for differentiating metastases from primary carcinomas of the lung.

Key Words: Differential diagnosis, Metastasis, Multiple lung tumor, Gene mutation, p53.

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The lung is one of the most common organs to which many carcinomas, including lung carcinomas, metastasize.[1] The incidence of multiple lung cancers in clinical reports ranges from 1 to 7%.[2] Therefore, the differential diagnosis of pulmonary nodules is important. When a clinician identifies multiple synchronous lung tumors, differentiating multicentric lung cancers, a single lung cancer with intrapulmonary metastases, or pulmonary metastases from primary cancer in different organs can often be difficult. When we need to differentiate between primary and metastasis for the diagnosis of multiple lung lesions, it is therefore useful to analyze the patterns of some genetic alterations.

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CASE REPORT

A 61-year-old Japanese woman underwent a total thyroidectomy for a left thyroid cancer (pT1N0M0, stage I) in 2004. The lesion showed papillary growth histologically, and was immunohistochemically positive for thyroglobulin. The next year, the patient underwent a partial mastectomy for a right breast cancer (pT1cN0M0, stage I). Microscopically, the tumor consisted of a well demarcated round nodule with focal invasion to adipose tissue in the breast (Figure 1). Neither estrogen (ER) nor progesterone receptors were immunohistochemically detected in the tumor cells. After 8 months, a follow-up chest computed tomographic scan revealed two small nodules in the left lower lobe (Segmentum basale laterale and Segmentum basale posterius; S9 and S10, measuring 10 mm and 8 mm, respectively). The computed tomography findings of the nodule in S9 revealed a solid density with a regular margin (Figure 2*A*). The findings of S10 revealed a solid density with a focal irregular margin (Figure 2*B*). We clinically diagnosed those lesions as metastatic lung cancer from the treated malignant lesions. The patient underwent a partial pulmonary resections by using a video assisted thoracoscopic approach for the malignant lesions on December 4, 2006. Microscopically, the tumor cells were cuboidal to columnar shaped cells growing along the alveolar walls in the S10 (Figure 3*A*). The immunohistochemical staining was positive for thyroid transcription factor 1 and P53. It was negative for thyroglobulin and ER. The nodule was pathologically diagnosed as a primary lung cancer (type B disease based on the Noguchi's classification[3]). But another tumor in the S9 was a poorly differentiated subtype of adenocarcinoma with intracytoplasmic mucin droplets (Figure 3*B*), and immunostaining was negative for

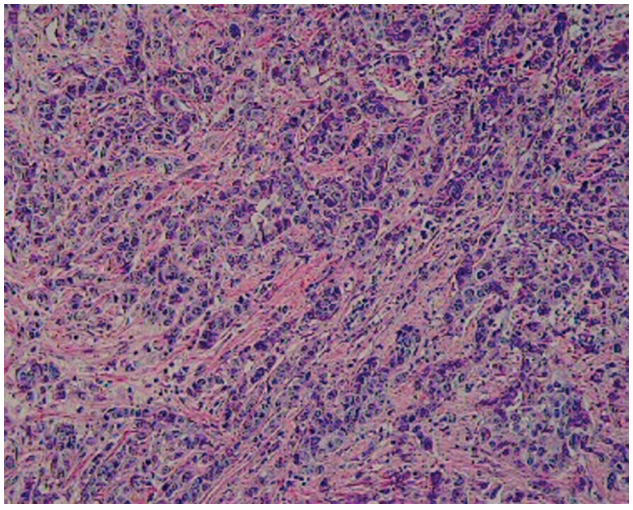

FIGURE 1. Microscopic findings of the right breast tumor reveal a ductal cancer cell type, and growth in well-defined nests (H&E stain, ×200).

ER, progesterone receptor, thyroid transcription factor 1, and thyroglobulin. It was positive for P53. In particular, it was not possible to judge the S9 nodule morphologically by microscopic examination to see whether it was a primary lung cancer or a metastatic lesion from the treated breast cancer. Direct sequencing mutation analyses of *p53* and the *K-ras* gene were performed for the differential diagnosis by the following methods. After we obtained consent from the patient, DNAs were purified from the tumor tissue. About six 10 μm sections were cut from the formalin-fixed block. The slices were deparaffinized with xylene, and genomic DNA were extracted from the tumor and nontumor samples using the DNeasy kit (Qiagen, Valencia, CA). Exons 5, 6, 7, and 8 of the *p53* gene were amplified by polymerase chain reaction (PCR) using specific oligonucleotide primers. The primers used for the amplification of exons were designed based on the published sequences.[4] The DNA was subjected to 40 cycles of PCR (95, 58, and 72°C for 0.5, 0.5, and 0.5 minutes, respectively) in PerfectShot Ex *Taq* (Takara Bio Inc. Japan). The PCR products were sequenced directly using a BigDye Terminator Cycle Sequencing Kit and an Applied Biosystems sequencer model 310 (Applied Biosystems, Foster City, CA). A different pattern of the distribution of the *p53* mutation in the two nodules was observed (Figure 4*A, B*), and the same pattern of the distribution of the *p53* mutation was observed in the DNA samples of the S9 nodule and the treated breast cancer. The mutation was detected in exon 6 (Figure 4*B, C*). These tumors had a C-to-T transition at codon 213. However, the *K-ras* mutation was not detected in any of the tumor samples. Therefore, we diagnosed the S9 nodule to be metastatic pulmonary carcinoma from the treated breast cancer based on the results of the *p53* mutation analysis.

DISCUSSION

Cancers of similar histologic types in different organs often share similar histologic, and even similar immunohistochemical features. Therefore, it would be expected that the patterns of some genetic alterations may serve as a discrimination marker of multiple lung lesions. The *p53* gene is localized on chromosome 17 (short arm, 17p13), a region that is frequently deleted in human cancer. It is one of the tumor suppressor genes, and codes for a 53-kDa nuclear protein. Mutations of the *p53* gene at a highly conserved sequence and alteration in the expression of the P53 protein are frequently present in human malignancies. In addition, a *p53* mutation is one common genetic change in nonsmall cell lung cancer. Chiba et al.[5] reported that mutations changing the *p53* coding sequence were found in 23/51 (45%) nonsmall cell lung cancer specimens, but not in the corresponding normal lung, and they were distrib-

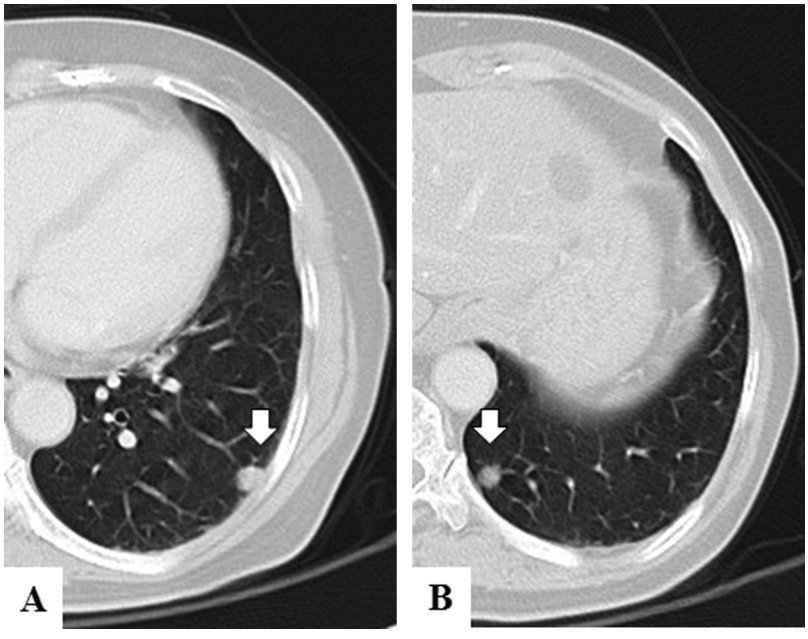

FIGURE 2. Chest CT image of the left lower lobe (S9) shows a solid density with a regular margin, and the size is a 10 × 10 mm (*A*). CT image of the left lower lobe (S10) shows a solid density with a focal irregular margin on the lung window, and the size is a 8 × 8 mm (*B*).

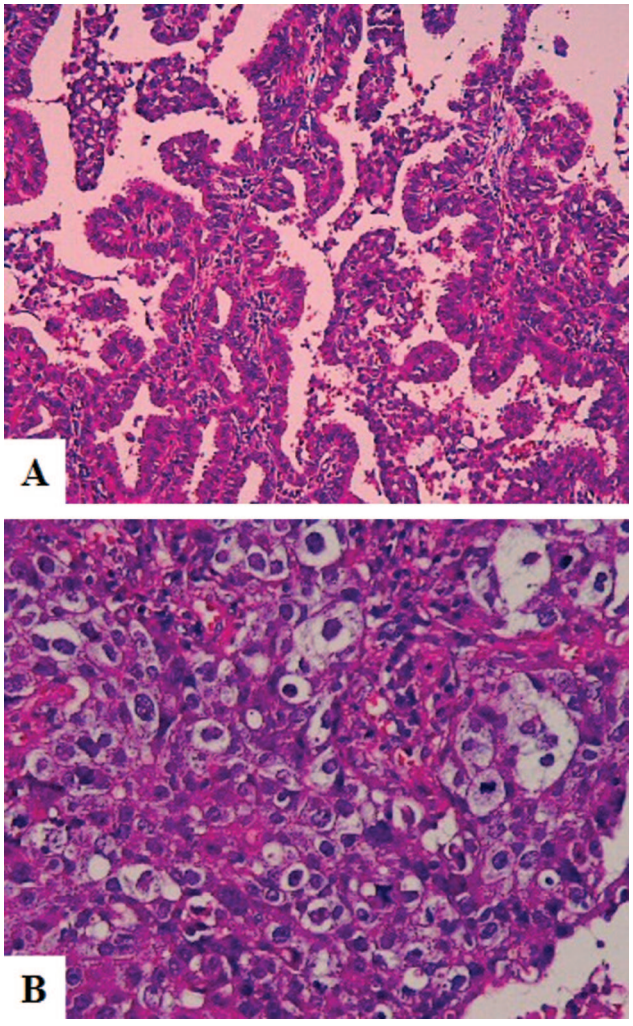

FIGURE 3. *A,* Microscopic findings of the resected tumor in the S10 reveal cuboidal to columnar shaped cells growing along the alveolar walls (H&E stain, *A* ×200). *B,* Microscopic findings of resected tumor in the S9 reveal a poorly differentiated subtype of adenocarcinoma with intracytoplasmic mucin droplets (H&E stain, *B* ×400).

uted between codons 132 and 283. In breast cancer, the *p53* mutation rate is about 20%. In addition, the *p53* mutation has been demonstrated to usually precede metastasis, while it is also conserved in metastases. Therefore, the analysis of the *p53* mutation pattern can be used as a clonal marker for the differential diagnosis of multiple cancers.[6]

In 1992, Oda et al.[6] reported the *p53* mutation pattern to be useful for distinguishing a primary tumor from metastasis and/or a second primary tumor from a recurrence in hepatocellular carcinomas. With other organs, the mutation pattern has been used to establish a differential diagnosis in ovarian cancers,[7] urothelial carcinomas,[8] and head and neck cancers.[9]

With respect to lung cancer, a comparison of *p53* mutations between primary lung cancer and metastatic lung tumors, primary lung tumors and metastases to other organs,[10] or second primary lung cancer and metastasis[1] has

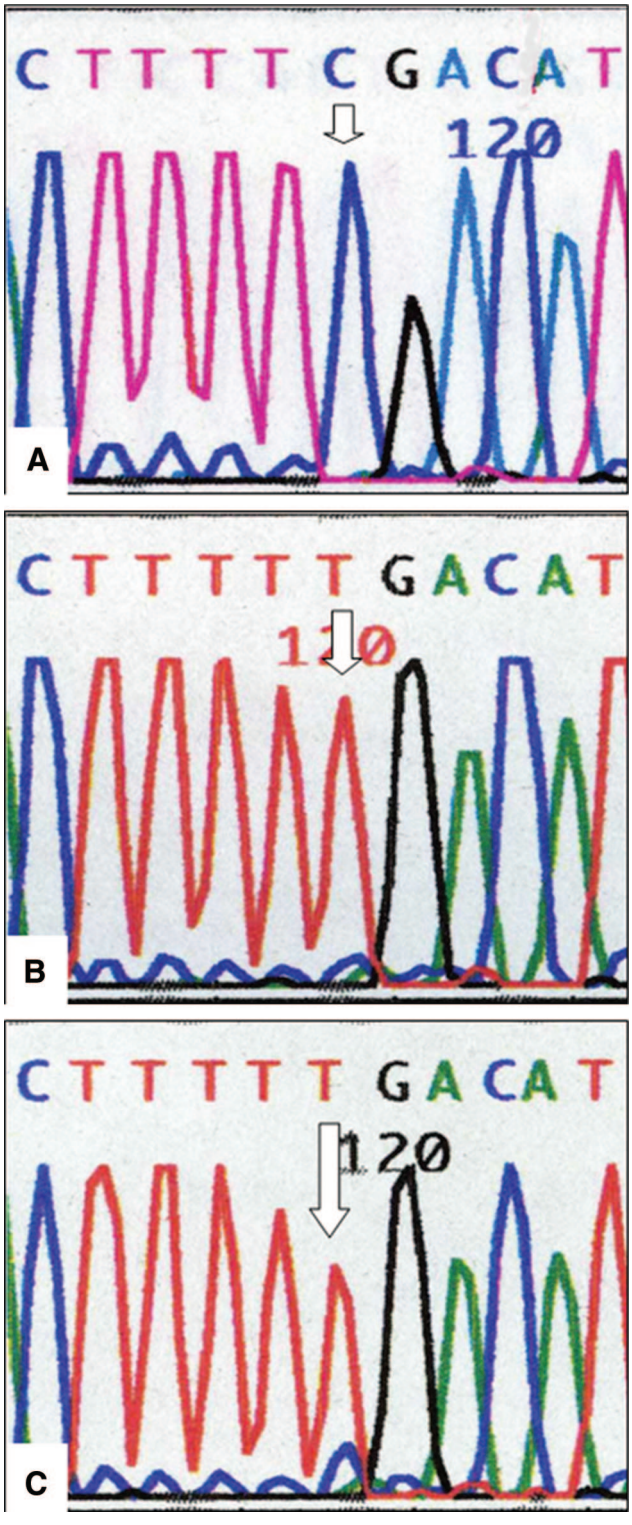

FIGURE 4. A sequence analysis of exon 6 from the tumor tissues. Codon 213 (the arrow) in the lung tumor of S10 is the normal base sequence (*A*). The arrow indicates a point mutation (codon 213; CGA→TGA) in the lung tumor of S9 (*B*). The arrow indicates the point mutation (codon 213; CGA→TGA) in the breast cancer (*C*).

been reported. These authors used PCR-single-strand conformation polymorphism as the method for performing a gene mutation analysis. The DNAs with mutations and those without mutations were able to be separated using *Taq*I (Takara Bio Inc., Japan) of the restriction enzyme. The band of mutated DNAs in polyacrylamide gels were cut out and then were further analyzed for their nucleotide sequences by the direct sequencing method (Fig. 4*A–C*).

In conclusion, when pulmonary tumors are observed in patients with a history of cancer, the differentiation between metastasis and primary lung cancer thus plays a crucial role in selecting the appropriate therapy for such patients. Assuming that we need to differentiate between primary and metastasis for the diagnoses of multiple lung lesions, a mutation analysis of the p53 gene is thus considered to be a good modality for differentiating metastases from primary carcinomas of the lung.

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